

**A practical manual on**  
**Fundamentals of Plant Breeding**

**AGP: 212 Credits: 3 (2+1)**  
**Semester III B.Sc. (Agriculture)**



**2020**

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**Syllabus:**

Plant Breeder's kit, Study of germplasm of various crops. Study of floral structure of self-pollinated and cross pollinated crops. Emasculation and hybridization techniques in self & cross pollinated crops. Consequences of inbreeding on genetic structure of resulting populations. Study of male sterility system. Handling of segregation populations. Methods of calculating mean, range, variance, standard deviation, heritability. Designs used in plant breeding experiments, analysis of Randomized Block Design. To work out the mode of pollination in a given crop and extent of natural out-crossing. Prediction of performance of double cross hybrids.

**Name of Student** .....

**Roll No.** .....

**Batch** .....

**Session** .....

**Semester** .....

**Course Name :** .....

**Course No. :** .....

**Credit** .....

**Published: 2020**  
**No. of copies: .....**  
**Price: Rs.**  
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**CERTIFICATE**

This is to certify that Shri./Km. ....ID No.....has completed the practical of course.....course No. .... as per the syllabus of B.Sc. (Hons.) Agriculture/ Horticulture/ Forestry ..... semester in the year.....in the respective lab/field of College.

Date:

Course Teacher

## INDEX

Ex. No.	Date	Topic	Page No.
1.		Plant Breeder's Kit	
2.		Reproduction in plants	
3.		Classification of crops based on mode of pollination	
4.		Mode of pollination in crop and extent of natural out-crossing	
5.		Floral Biology of crops	
6.		Hybridization technique in Rice	
7.		Hybridization technique in Maize	
8.		Hybridization technique in Pigeonpea	
9.		Pollen Viability Test	
10.		Methods of calculating mean, range, variance and standard deviation	
11.		Assessment of Genetic variability	
12.		Analysis of Randomized Block Design	
13.		Estimation of heterosis and inbreeding depression	
14.		Prediction of performance of double cross hybrids	
15.		Induction of Mutation	
16.		Induction of Polyploidy	
17.		Planting of a crossing block and raising of F <sub>1</sub> and F <sub>2</sub> generation	
18.		Data collection and record keeping	

**Exercise No. 1**

**Objective: To know tools in Plant Breeder's kit and their uses**

**Materials required:**

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**Plant Breeder's kit**

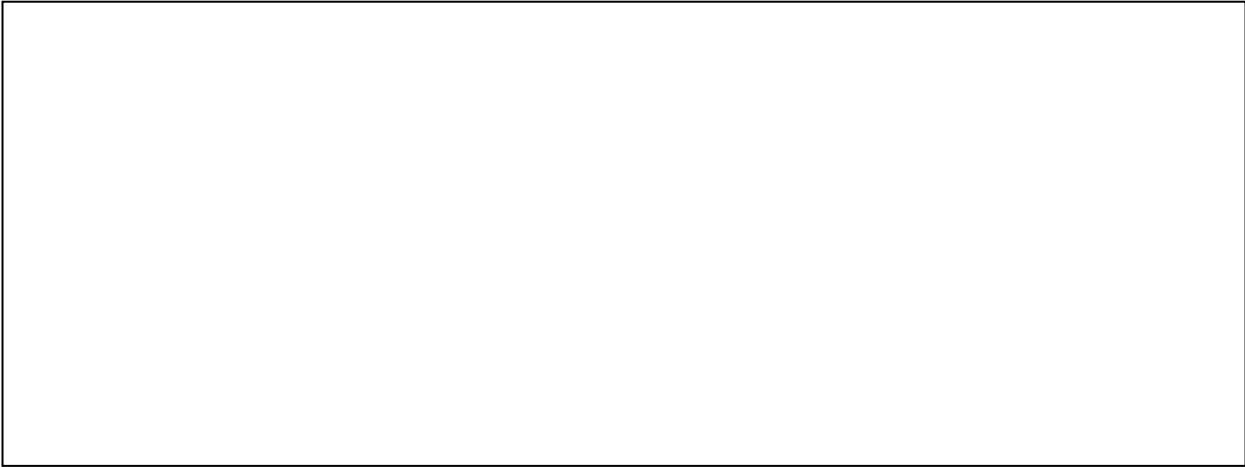
<b>S. No.</b>	<b>Items</b>	<b>Purpose</b>
1		..... ..... .....
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## Exercise No. 2

**Objective: To study about sporogenesis and gametogenesis**

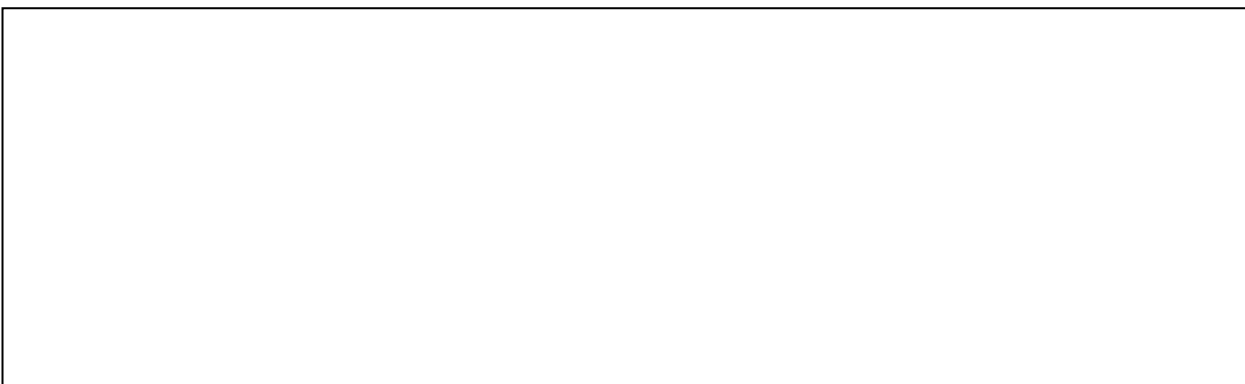
**1. Draw a well-labelled diagram of a mature embryo sac**



**2. With a neat, labelled diagram, describe the parts of a typical angiosperm ovule**



**3. Describe the structure of a pollen grain with the help of a diagram**









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**Observations:**

Name of Crop	Inbreeding Depression (%)	Mode of pollination

**Extent of out crossing:**

**Procedure:**

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**Observation:**

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**Result:**

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## Exercise No. 5

**Objective: To study the floral structure of self-pollinated and cross-pollinated crops**

**Materials required:** Scissors, forceps, plant inflorescence of rice, wheat, maize, pigeonpea, dissecting microscope

### A. Self-pollinated crop

**Rice (*Oryza sativa*):**

**Floral diagram of rice spikelet**

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**Wheat (*Triticum aestivum*):**

**Floral diagram of wheat spikelet**

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### B. Cross-pollinated crop

**Maize (*Zea mays*):**

**Diagram of Male inflorescence**

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**Diagram of Female inflorescence**

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**Diagram of Longitudinal section through female inflorescence (*cajan*)**

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### C. Often- cross pollinated crop

**Floral diagram of Pigeonpea (*Cajanus*)**

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**Observations:**

<b>Date of emasculation</b>	
<b>Time of emasculation</b>	
<b>Date of pollination</b>	
<b>Time of pollination</b>	
<b>Seed set Percentage</b>	

**Precautions:**

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**Observations:**

<b>Date of emasculation</b>	
<b>Time of emasculation</b>	
<b>Date of pollination</b>	
<b>Time of pollination</b>	
<b>Seed set Percentage</b>	

**Precautions:**

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**Exercise No. 8**

**Objective:** Study the emasculation and pollination technique in pigeonpea

**Materials Required:** Scissors, needle, butter paper bag, pencil, tag, petri plate, brush etc.

Scientific name: ..... Chromosome Number – 2n = .....

**Emasculation and Pollination technique**

**Procedure:**

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**Observations:**

<b>Date of emasculation</b>	
<b>Time of emasculation</b>	
<b>Date of pollination</b>	
<b>Time of pollination</b>	
<b>Seed set Percentage</b>	

**Precautions:**

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**Observations:**

Slide No.	Total number of pollen grains	Number of pollen grains stained	Number of pollen grains unstained	Pollen fertility (%)

**Pollen fertility (%) = Total number of stained pollen/total number of pollen grains) × 100**

**Pollen fertility of A, B and R Lines of .....**

Lines	Number of pollen grains		Total number of pollen grains	Pollen fertility (%)
	Unstained	Stained		
A				
B				
R				

**Results:** .....

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**Precautions:** .....

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**Numerical 2.** From the analysis the data generated are given below

Environmental variance = 1.96

Phenotypic variance = 9.82

Population mean = 55.75

Calculate the Heritability, Genotypic coefficient of variation and phenotypic coefficient of variation.

**Solution:** .....

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**Numerical 3.** Calculate the narrow sense and broad sense heritability if

$V_e = 10.8$        $V_E = 3.5$        $V_D = 8.8$        $V_P = 30.7$

**Solution:** .....

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**Objective: To induce mutation in crop plants**

**Materials required:** .....

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**Procedure:** .....

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**Objective: To induce polyploidy in crop**

**Materials required:** .....

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**Procedure:** .....

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**Observations:**

S. No.	Parameters	
1.	Material treated	
2.	Colchicine concentration used	
3.	Method of application	
4.	Duration of treatment	
5.	Proportion of expected polyploids	
6.	Size of stomata and count	

**Result:** .....

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## Exercise No. 17

**Objective:** To learn the method of planting a crossing block,  $F_1$  and  $F_2$  generations.

**Question:** A set of ten chickpea/wheat varieties of is to be planted in a crossing block. Prepare the field layout and outline the necessary precautions.

### Layout Plan

A large empty rectangular box with a thin black border, intended for the student to draw a field layout plan for a crossing block of ten chickpea/wheat varieties. The box is currently blank.

**Question 2:** F<sub>1</sub> seeds of 5 crosses of ..... are available. Record the data in the table.  
 Prepare the field layout plan and describe steps necessary to produce F<sub>2</sub> generation.

**Important Characters of F<sub>1</sub> plants**

S. No.	F <sub>1</sub>	Characters							
1									
2									
3									
4									
5									

**Layout plan of F<sub>2</sub>**

**Important Characters of F<sub>2</sub> plants**

S. No.	Plant no. of each F <sub>2</sub>	Character 1	Character 2	Character 3	Character 4
1					
2					
3					
4					
5					

**Exercise No. 18**

**Objective:** To collect data on various characters of the plant material under study and maintain a record.

**Materials required:**

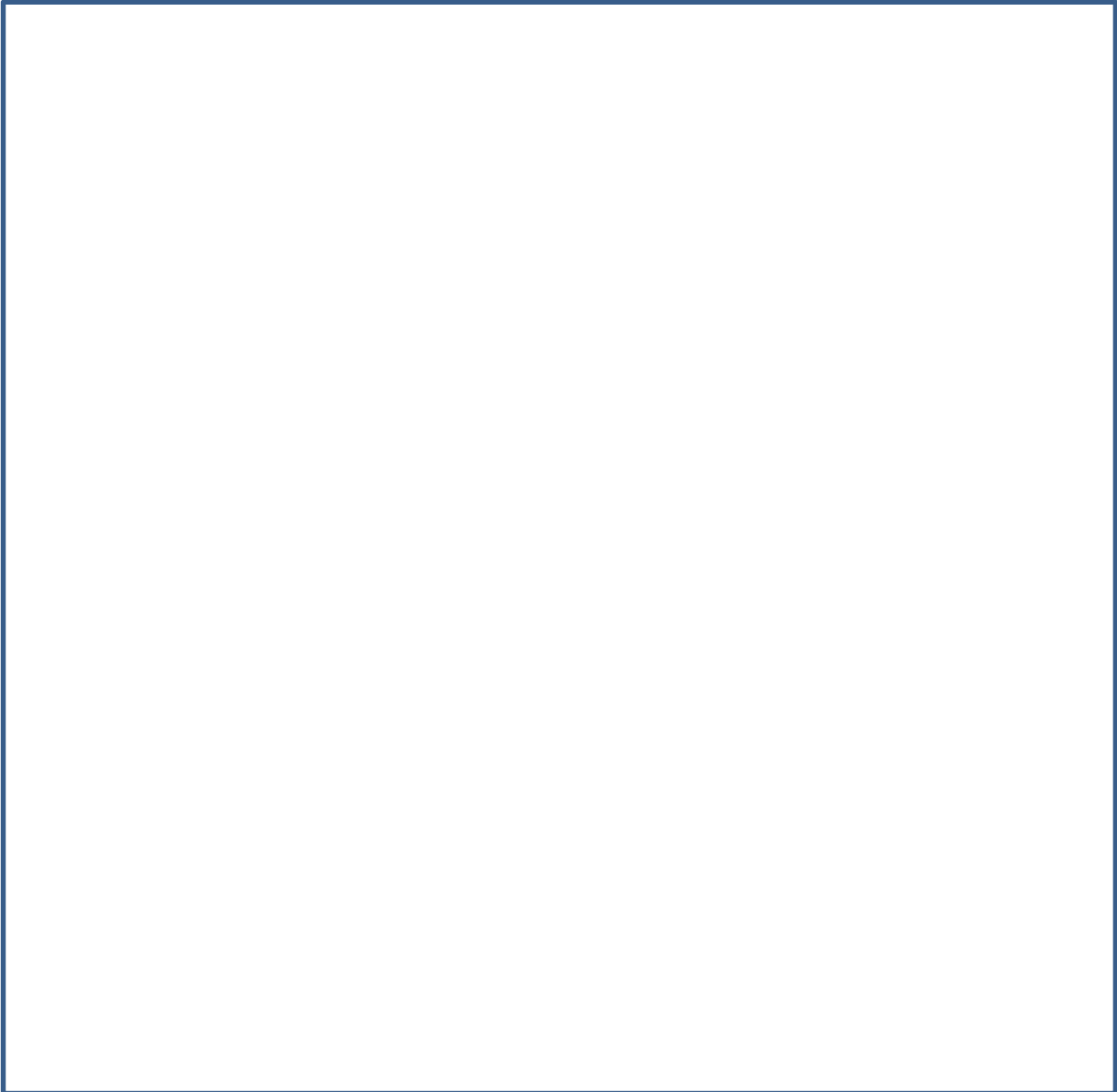
**A. Yield Trial**

**i) First Page**

- a. Number and title of the project: .....
- b. Season of raising the crop: .....
- c Unit under which the trial is being conducted: .....

**ii) Second page**

- a. A full plan of the field showing the location of the trial with the approach path. (North East directions should be specified)



**iii) Third Page**

- a) Plan of the experiment: .....
- b) Experiment details: .....
  - 1. Name of the experiment: .....
  - 2. Season: .....
  - 3. Number of variants: .....
  - 4. Design of the experiment: .....
  - 5. Replication: .....
  - 6. Size                                  of                                  the                                  plot:  
.....
  - 7. Spacing: .....
  - 8. Date of sowing/planting: .....
  - 9. Date of harvest: .....
  - 10. Name of the Principal Investigator: .....

**iv) Fourth page**

Details of cultural practices followed for the plot/ field

- a. Date of ploughing: .....
- b. Date of layout of the trial: .....
- c. Manurial schedule adopted
  - Basal                                  : .....
  - Topdressing                                  : .....
- d. Irrigation schedules with date from life irrigation onwards: .....
- e. Plant protection schedules followed: .....
- f. Details of inter-cultural operations A (hoeing, weeding, and earthing up etc.):  
.....
- g. Date of harvest: .....
- h. Duration of processing till storage: .....
- i. Rainfall received during the crop growth: .....



PLANT BREEDER'S KIT

Plant breeding experiments require numerous tools and instruments for selfing, crossing, emasculation, etc. activities.

**Fine pointed forceps:** It is used for incising the floral buds and for removing the anthers from it. E.g. Tobacco, sesamum etc.

**Small/ curved scissor:** For cutting the small florets in cereals and small flowers in the crops like lucerne, guar etc.

**Long straight scissor:** Used for clipping, cutting the vegetable parts and large size floral parts in cereals like wheat, sorghum, bajra, and tobacco.

**Sharp pointer:** Used for incising the floral parts and for removing the anthers from the crops like bajra.

**Eye lens or magnifying lens:** Used for observing the reproductive parts to confirm that there should not be any part of the anther left on the stigma or stigma is free from any foreign pollens.

**U-pins (u- clips):** Used for fastening the bags on earheads or flowers to keep the bag in proper position.

**Paint brush:** Used for transferring the pollen grains in crops like Castor, Sorghum etc. Advantage: Without injuring to stigmas or pollens, pollination is accomplished very smoothly.

**Pencil:** Used for writing field labels or field bags. Sometimes it is also used for emasculating the sorghum flowers. Compared to ink or ball pen writing, pencil writing should be preferred as it will not erase or spread during rains, dew or under intense light.

**Meter scale:** Used for plant measurement in the field.

**Needles:** Required to open small buds and separating the floral parts.

**Washing bottle:** Used for filling sterilizing agent like alcohol or spirit to sterilize the scissors, pointers, forceps and brush during crossing work.

**Wire ring and smooth thread:** Used for selfing in crops like Cotton, Okra, Sesamum etc. Thread is used for fastening(tying) the bud, while ring is inserted in axis of flowers to identify it. Compared to bags, this method is more convenient and cheaper

**Small white tag:** Used for identifying the internal flower or a small twig during crossing programme. The detailed information about crossing is written on it with pencil and then it is inserted on pedicel or peduncle e.g. Cotton, Bajra, Wheat, Sorghum, Sesamum etc.

**Soda straw tubes:** Used for protecting the emasculated or pollinated flower buds of cotton, tobacco etc. **Advantage:** compared to paper bags it is very convenient, easy and cheaper method of selfing and crossing.

**Waxy threads:** Used for fastening(tying) the luggage labels on the plants.

**Luggage labels (white or yellow):** Used for tagging the large sized plants like Tur or Castor while rouging or during selection.

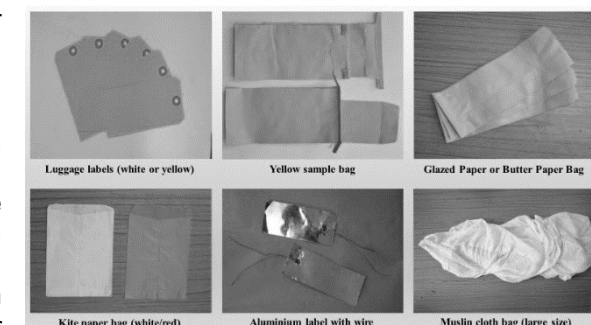
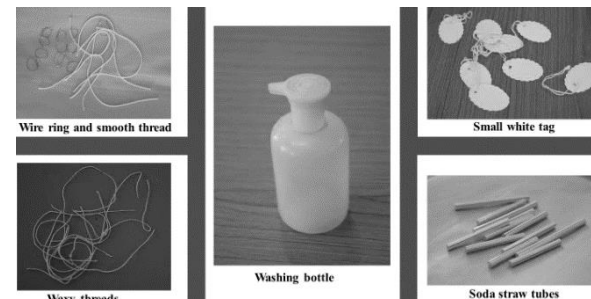
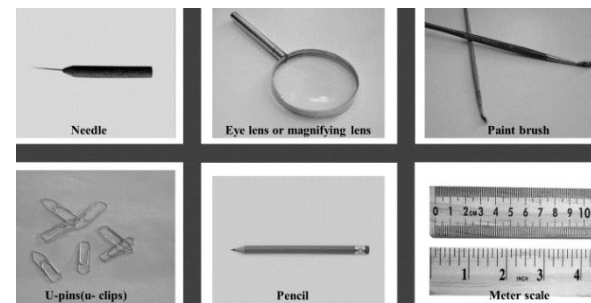
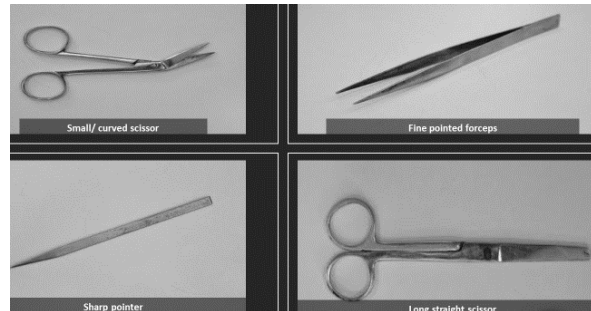
**Aluminium label with wire:** Used for tagging the flowers in fruit crops or tree species after crossing. It is also used for identification of selected trees.

**Muslin cloth bag (large size):** Used to cover the whole plant while selfing or crossing in the crops like Chilly, Brinjal etc. In large sized plants like Tur it can be used for protecting individual branch also.

**Yellow sample bag:** Used for storing the crossed seeds in small quantity.

**Glazed Paper or Butter Paper Bag:** Used for selfing Bajra, Wheat, Sorghum, Castor etc.

**Kite paper bag (white/red):** Used for protecting small size flowers of Pulses, Oilseeds and Other food crops during selfing and crossing



## REPRODUCTION IN PLANTS

**Sporogenesis:** Process of production of microspores and megaspores is known as sporogenesis.

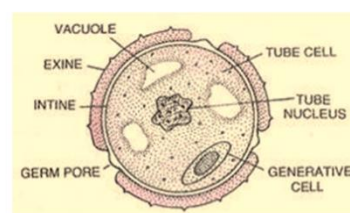
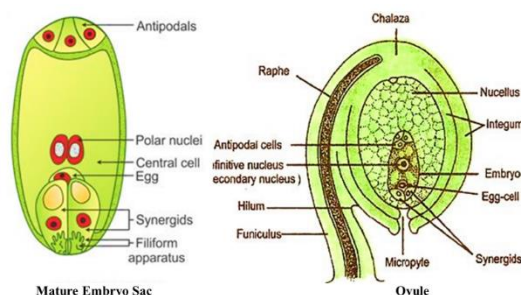
**Microsporogenesis:** The sporophytic cells in the pollen sacs of anther which undergo meiotic division to form haploid i.e., microspores are called microspore mother cell (MMC) or pollen mother cell (PMC) and the process is called microsporogenesis.

**Megasporogenesis:** A single sporophytic cell inside the ovule, which undergo meiotic division to form haploid megaspore, is called megaspore mother cell (MMC) and the process is called megasporogenesis.

**Gametogenesis:** The production of male and female gametes in the microspores and megaspores, respectively, is known as gametogenesis.

**Microgametogenesis:** Microgametogenesis refers to the production of male gametes or sperm. On maturation of the pollen, the microspore nucleus divides mitotically to produce a small generative and a large vegetative or tube nucleus.

**Megagametogenesis:** The nucleus of the functional megaspore divides mitotically to form two nuclei which move to the opposite poles, forming the 2-nucleate embryo sac. Two more sequential mitotic nuclear divisions result in the formation of the 4-nucleate and later the 8-nucleate stages of the embryo sac. There is a characteristic distribution of the cells within the embryo sac. Three cells are grouped together at the micropylar end and constitute the egg apparatus. The egg apparatus, in turn, consists of two synergids and one egg cell. Three cells are at the chalazal end and are called the antipodals. The large central cell, as mentioned earlier, has two polar nuclei. Thus, a typical angiosperm embryo sac, at maturity, though 8-nucleate is 7-celled. (The exact number of nuclei and their arrangement varies from one species to another.)



Section of a 2 celled pollen grain of an angiosperm

## CLASSIFICATION OF CROPS BASED ON MODE OF POLLINATION

Pollination refers to the transfer of pollen grain from anthers to stigmas. Pollen from an anther may fall on the stigma of the same flower leading to self-pollination or auto gamy. Sometimes pollen from an anther may fall on the stigma of another flower of different plants leading to cross pollination or allogamy. Sometimes pollen from an anther fall on the stigma of the anther flower of same plant leading to the geitonogamy.

**Self-Pollination:** It is transfer of pollens from and to the stigma within the same flower, is always found in bisexual flower. In most of these species self-pollination is not complete and cross- pollination may occur up to 5%. There are various mechanism/ contrivances that promote/facilitate self-pollination.

**Bisexuality:** Male and female sexual organs present in the same flower e.g wheat, rice, groundnut, etc.

**Homogamy:** Male and female sexual organs mature at the same time e.g wheat, groundnut, etc.

**Cleistogamy:** In this condition flowers do not open at all and ensure complete self-pollination. e.g oat, barley, wheat, grasses, etc.

**Chasmogamy:** In some species, flower open but only after pollination has taken place. e.g. Barley, wheat, oat, and many cereals.

In crop like Tomato and Brinjal stigma are closely surrounded by anthers, hence pollination occurs after opening of flower but the position of anther in relation to stigma ensure self-pollination.

### Genetic Consequences of Self-pollination:

- i) It leads to a very rapid increase in homozygosity; therefore, self-pollinated species are highly homozygous in nature.
- ii) Self-pollinated species do not show inbreeding depression, exhibit considerable heterosis.

**Cross-Pollination:** The transfer of pollen from a flower to the stigma of the other flower of different flower plant. In cross pollinated species pollination may be brought about by wind, water insect or animals. Wind (anemophily), water (hydrophily), insect (entomophily) and animal (zoophily). In most of the cross-pollinated sp. viz., bajara, maize, sunflower, alfalfa, castor, cross pollination is not complete, and self-pollination may occur 5-10%. There are several mechanism contrivances that facilitate cross pollination.

**Dicliny (Unisexuality):** It is a condition in which flower is either staminate or pistilate.



**Monoecy:** Staminate and pistillate flowers occur in the same plant either in the same inflorescence. e.g. Mango, banana, coconut or in the separate inflorescence. e.g. Maize, Cucurbit, Strawberry, etc.

**Dioecy:** The male and female flowers are present on different plants i.e. the in such species are male or female i.e. sex is governed by a single gene. e.g. Papaya, hemp, date, palm, etc.

**Dichogamy:** Anther and stigma of hermaphrodite flower mature at different time, facilitating cross pollination.

**Protogyny:** Gynoecium matures earlier than the androecium e.g. Bajara.

**Protandry:** Androecium matures earlier than gynoecium. e.g. marigold, maize, cotton, etc.

**Heterostyly:** Different length of style and filaments e.g. Linseed.

**Herkogamy:** Presence of physical barrier or mechanical obstacles between the anther and stigma ensures cross pollination. e.g. Rui (*Calotropis gigantea*).

In lucerne or alfalfa stigma are covered by waxy film and it does not become receptive unless this waxy film is broken by honeybees.

A combination of two or more of the above mechanism may occurs in some species, e.g. Maize, - Monoecy and Protandry.

**Self –Incompatibility:** It refers to the failure of pollen from a flower to fertilize the same flower or other flowers on the same plant. It may be saprophytic or gametophytic. e.g. mustard, tobacco, sunflowers, reddish.

**Male Sterility:** It refers to the absence of functional pollen grains in hermaphrodite flower.

**Genetic Consequences of Cross Pollination:** 1) It preserves and promotes heterozygosity in population. 2) Cross pollinated species shows inbreeding depression and considerable heterosis. 3) Usually hybrid and synthetic without reducing heterozygosity.

**Often Cross Pollination:** In this type plants are self-pollinated; however, the extent of cross pollination often exceeds 5 to 50 % such species are generally known as often cross-pollinated species. e.g. Jawar, Cotton, Safflower, Arhar, etc. The genetic architecture of such crop is intermediate between self- and cross-pollinated crops and breeding methods suitable for both of them may be profitably applied.

### MODE OF POLLINATION IN CROP AND EXTENT OF NATURAL OUT-CROSSING

There are some natural mechanism favoring a particular type of pollination which are of great use to differentiate pollinating system in crop plants. However, some other methods are also available which can be appropriately applied with natural devices for this purpose. There are several approaches to work out the mode of pollination in a given crop:

**Morphological examination of flowers:** Mechanism like dioecy, monoecy, protogyny, protandry and cleistogamy are easily detected. They indicate the mode of pollination.

Cleistogamous condition:	Self-pollinated
Chasmogamous condition:	Self-pollinated
Homogamy:	Self-pollinated
Dichogamy:	Cross-pollinated
Herkogamy:	Cross-pollinated

**Space isolation:** If a single plant is raised in isolation normal seed setting will indicate self-pollination while erratic or no seed set means flowers need pollen from other sources. Thus, cross pollination can easily be identified by isolation planting.

**Inbreeding depression (Effects of selfing):** If selfing of plants leads to considerable loss of vigor, cross pollination may be anticipated. Conversely, little or no loss of vigor indicate self-pollination in plants.

**Objective: To work out the extent of out crossing:** The amount of cross-pollination is determined by planting two strains of the concerned species in a mixed stand. One of these two strains is homozygous for a dominant character, preferably an easily recognizable seeding or other phenotypic character, while other strain is recessive for that character. The recessive strain may be planted preferably in a polycross nursery in isolation along with dominant strain. Collect the seeds produced on the recessive strain and grow them in the next generation. The percentage of plant carrying the dominant allele of the character represents the percentage of cross-pollination.

## FLORAL BIOLOGY OF CROPS

### Rice (*Oryza sativa*):

**Panicle:** The inflorescence of rice plant, borne on terminal shoot and thus called as panicle. It is determinate type and at maturity, it is droopy in nature.

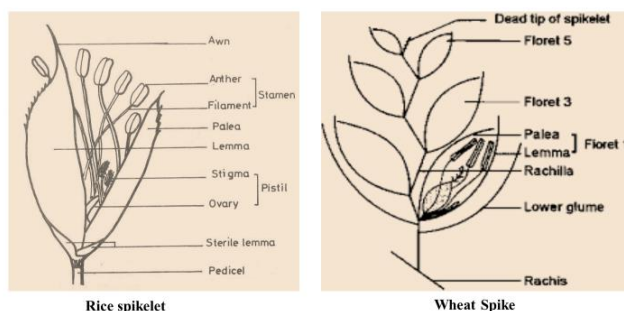
**Spikelet:** A spikelet is the floral unit and consists of two sterile lemmas, a lemma, a palea and the flower.

Its parts are:

**1. Lemma:** It is a 5- nerved hardened bract with a filiform extension (of the middle nerve) known as awn.

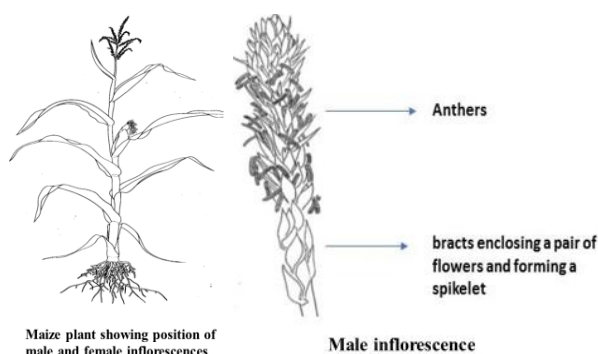
**2. Palea:** It is a 3- nerved bract slightly narrower than lemma.

**3. Flower:** It consists of 6 stamens with two-celled anthers and a pistil with one ovary and two stigmas. The pistil contains one ovule.



**Wheat (*Triticum aestivum*):** The inflorescence of wheat is a spike bearing two opposite rows of lateral spikelets and a single terminal spikelet on the primary axis. The unit of spike is called spikelet. Two to five florets are born in each spikelet, subtended by a pair of glumes. Each floret contains three anthers and a pistil bearing two styles each with feathery stigma and two ovate lodicules which are modified perianth structure. Florets at anthesis are forced open by swelling of the lodicules. A wheat grain is caryopsis, a small dry, indehiscent, one seeded fruit with a thin pericarp consisting of a germ or embryo and an endosperm.

**Maize (*Zea mays*):** It is monoecious plant bearing male flower are the growing tip as tassel and female flower at the axial of the leaf on the shoot. The tassel is terminal with staminate flowers in several roots. Each pair of flowers consist of sessile and pedicillate spikelet. Each spikelet contains two similar glumes. The flower contains membranous palea with three stamens and two lodicules. The female inflorescence is a spadex known as cob or ear. Spikelets are in pair. Each spikelet having two flowers, the lower one is reduced to lemma and palea is non-functional, while upper one contained knob shaped ovary surrounded by broad lemma and thin palea. One carpel is provided with long silky hair, which behaves as style and style stigma throughout the length.



**Pigeonpea (*Cajanus cajan*):** The inflorescence is an axillary raceme often forming a terminal panicle. The size of inflorescence varies in different types. The flowers are distinctly papilionaceous. Individual flower consists of a calyx with five sepals and corolla with a standard petal, two wings petals, and a keel petal. Stamens are 10, diadelphous (9+1), flattening towards the base, tapering towards the top, and geniculate near the base. The anthers are ellipsoid, dorsifixed, and light or dark yellow in color. Of the 10 stamens, four have short filaments and six, including the odd posterior one, have long filaments. The odd stamen has a groove for the passage of nectar that is secreted from the base of the filaments. The long stamens are antisepalous and the short ones antipetalous.

## HYBRIDIZATION TECHNIQUE IN RICE

Scientific name: *Oryza sativa*

Chromosome Number –  $2n = 24$

Family: Poaceae

In rice anthesis commences shortly after emergence of panicle. Spikelet at the tip bloom first and proceed downwards.

Anthesis time is 08:00-10:00 a.m. Each spikelet remains open 30 minutes and then closes. The anther dehiscence takes place immediately after opening of the spikelet. Receptivity remains for one day.

### Emasculation and Pollination technique

#### I. Clipping method

1. One day prior to anthesis, in the evening, top of 1/3<sup>rd</sup> and bottom 1/3<sup>rd</sup> portion in the panicle of desired female parent are clipped off by using scissors leaving the middle spikelet.
2. With the help of scissors top 1/3<sup>rd</sup> portion in each spikelet is clipped off in a slanting position.
3. The six anthers present in each spikelet are removed with the help of needle/forcep. (Care must be taken during emasculation for not to damage the gynoecium.
4. To prevent the contamination from the foreign pollen, the emasculated spikelets are covered with a butter paper bag.
5. In the next morning (usually 08:00 a.m.) the bloomed panicle from the desired male parent is taken or the pollens are

collected in a petri plate.

6. The top portion of the butter paper bag which was originally inserted in the emasculated female parent is now cut to expose the panicle.
7. The male panicle is inserted in an inverted position into the butter paper bag and sturned in both ways in order to disperse the pollen (or dusting of pollen can be done, if pollen is collected in a petri plate).
8. After ensuring the abundant dispersion of pollen, the opened butter paper bag is closed using pin. Coloured thread may be tied at the base of the panicle to identify the crossed one.

## II. Hot water method

1. An hour so before blooming (i.e. normally at 07:00 a.m.), the panicle is selected and underdeveloped and opened spikelets are removed.
2. Now, the tiller is bent over (carefully to avoid breaking) and the selected panicle is immersed in hot water contained in a thermos bottle at 40-44°C for a period of 5-10 minutes.
3. This treatment causes the florets to open in a normal manner and avoids injury
4. Then emasculation is done by removing the six stamens by fine forceps or needle and then dusting should be done.

## HYBRIDIZATION TECHNIQUE IN MAIZE

Scientific name: *Zea mays* Chromosome Number –  $2n = 20$  Family: Poaceae

**Materials Required:** Tassel bag, butter paper bag, ear bag, pencil, tag, petri plate, brush, scissors etc.

### Emasculation:

1. The tassels of the female plants are removed immediately as soon as appeared. The process is called as detasseling. It is always done in the morning.
2. Ear shoot which emerging from the leaf sheath is bagged 1 to 2 days below the tip of the previous day of pollination.
3. The tassels of selected male parents are also covered with bag on following day in the morning between 9.00 to 10.00 a.m. pollens from tassel bag is dusted over the silk of the female cob/ear.
4. The bag covered ear shoot is torn and bag from the male parent may be placed over the cob. Care should be taken to avoid contamination of silk with foreign pollens.

### Crossing technique

**Female parent:** a. Detassel and b. Cut the tip of the cob before the silks emerge and cover with a butter paper cover.

**Male parent:** a. Cover the tassel before anthesis begins or as soon as the tassel emerges.

When the silk emerges in the female parent in the form of a brush, pollination is done by transferring the freshly shed pollen cover form the male parent and inserting it over the cob of the female parent after removing the cover from the cob. The details like date of pollination, parentage and breeding programme

The details like date of pollination, parentage and breeding programme to be carried out are clearly written by water-proof pencil. The date or pollination will be one day later than the date of tasselling. Pollination should be completed within one week of silk emergence.

## HYBRIDIZATION TECHNIQUE IN PIGEONPEA

**Materials Required:** Scissors, needle, butter paper bag, pencil, tag, petri plate, brush etc.

**Scientific Name:** *Cajanus cajan*

Flowers start opening early in the morning in the summer and by noon during winter, continuous opening throughout the day. The length of time flowers remain open is influenced by the weather.

Stigma becomes receptive for pollination 68 hours before anthesis and remains in the same condition for about 20 hours after anthesis. Pollen in buds remain viable up to 24-28 hrs after dehiscence at room temperature (25-28°C, 50.6% humidity)

**Emasculation:** Tightly closed buds, approximately two-thirds the size of mature buds, should be emasculated. At the correct stage the bud should show a bright corolla color without any greenish hue. The selected bud should be firmly held between the thumb and the middle finger with the index finger used to support the flower. The curved side of the standard is held toward the crosser and the sepal covering the keel is removed. The corolla is opened by inserting one of the tips of a fine pointed forceps at the base of the keel and moving upward to the tip of the standard. The bud will open with slight pressure of the supporting index finger, and the well-developed yellow anthers can be removed from the staminal column with forceps.

**Pollination:** The stigma is receptive before anthesis thus pollination can be done immediately after emasculation. Pollen source buds from male parent should be collected between 8 and 10 am. These should be large, unopened buds in which the anthers will dehisce on the day collected. For selfing, cloth bags or fine-mesh nylon cloth bags are effective in excluding vector insects; cross-pollinated flowers can be identified with colored threads.

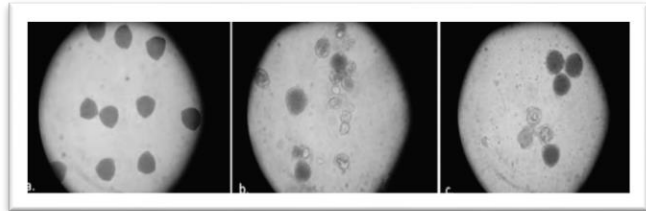
### POLLEN VIABILITY TEST

The branch of science, which deal with the study of pollen grains is called palynology. Pollen viability refers to the ability of a pollen grain to germinate and produce male gametes. Pollen grains of different crops are viable for a short period after release from anthers. Several chemical stains may be used for testing pollen viability such as acetocarmine, acetoorcein, potassium iodide, triphenyl tetrazolium chloride, Evan's blue etc.

**Preparation of Acetocarmine Stain (C<sub>22</sub>H<sub>2</sub>O<sub>13</sub>):** It is one of the most widely used stain for pollen study. A mixture of 4 ml glacial acetic acid and 55 ml of distilled water is boiled. A quantity of 2 g of carmine (according to the strength required) is added to 100 ml of the above mixture at about boiling point and then boiled for few minutes. After boiling, the contents are removed from the flame and allowed to cool and filtered in a clean bottle. The filtrate is reddish in colour and known as 2% acetocarmine. Ferric chloride or ferric acetate may be added if necessary, for deep staining and preservation.

#### Pollen viability test:

1. To determine the pollen fertility fully developed floral buds will be collected randomly from each plant and the anthers will be squashed in 2% aceto-carmine stain on a micro slide and examine under a light microscope using 100x magnification.
2. The viable pollen grains will be deeply stained, whereas dead pollen will be unstained and will be shriveled and irregular.
3. Five microscopic fields for each sample will be examined. Mean of the five microscopic fields will be calculated and the proportion of fertile pollens will be expressed in percentage.
4. Percent pollen fertility will be calculated as



Pollen Fertility (a) Fertile pollen; (b) Sterile pollen; (c) Partially fertile pollen

$$\text{Pollen fertility (\%)} = \frac{\text{Total number of stained pollen}}{\text{Total number of pollen}} \times 100$$

[Normal deeply stained pollen grains were counted as viable, while weakly stained were recorded as non-viable].

### MEAN, RANGE, VARIANCE AND STANDARD DEVIATION

**Mean:** defined as the sum of all the observation in a sample divided by their number and is denoted by  $\bar{y}$

$$\bar{y} = \frac{\sum y_i}{n}$$

Where  $\bar{y}$  = denoted the mean value,  
 $\sum y_i$  = Sum of the  $y_i$  observations

In case of frequency distribution, the mean is obtained by multiplying the observation by its frequency, adding the products and dividing by member of observations.

$$\bar{y} = \frac{\sum (fy_i)}{n}$$

Where  $\bar{y}$  = mean value,  
 $y_i$  = observations,  
 $fy_i$  = frequencies of observations,  
 $\sum$  = Summation and  
 $n$  = total number of observations.

**Range:** It is difference between the lowest and the highest values present in the observation of a sample

**Variance:** Variance is defined as the average of the standard deviation from the mean. It is estimated as:

$$\text{Variance (V)} = \frac{1}{n-1} \sum (y_i - \bar{y})^2$$

Where  $\bar{y}$  = mean value,  
 $y_i$  = observations,  
 $\sum$  = Summation and  
 $n$  = total number of observations.

**Standard deviation:** It is the square root of mean of the squares of the deviations measured from the mean. In other words, it is the square root of variance.

$$\text{Standard deviation (SD)} = \sqrt{\frac{1}{n-1} \sum (y_i - \bar{y})^2}$$

$$\text{Or SD} = \sqrt{\text{variance}}$$

**Standard error:** It is the measure of the mean difference between sample estimate of mean and the population parameter ( $\mu$ ).

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{N}}$$

**Coefficient of Variation:** It is the measure of variation which is independent of the unit of measurement is provided by the standard deviation expressed as percent of mean.

$$\text{Coefficient of Variation} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

### GENETIC VARIABILITY

The phenotype may be described according to a mathematical model to facilitate statistical analysis and interpretation. The phenotypic mean i.e.  $\bar{x}$  of a given genotype from the trial may be expressed as  $m$ ,

$$\bar{x} = \mu + g + e + ge \text{ where,}$$

$\bar{x}$  = phenotypic mean

$\mu$  = General population mean

$g$  = effect of genotype

$e$  = effect of environment

$ge$  = interaction between genotype and environment

#### Analysis of variance for genotypes grown in a replicated trial according to RBD

Source of variation	d.f.	Expectation of MS
Replications	$r-1$	$\sigma_e^2 + g\sigma_r^2$
Genotypes	$g-1$	$\sigma_e^2 + r\sigma_g^2$
Error	$(r-1)(g-1)$	$\sigma_e^2$
Total	$(rg)-1$	

$g$  and  $r$  are the number of genotypes and replications respectively; and  $\sigma_e^2$ ,  $\sigma_r^2$  and  $\sigma_g^2$  denote the variances due to error, replications and genotypes respectively.

Genotypic variance ( $\sigma_g^2$ ) = (MS due to genotypes – MS due to error)/ $R$

Phenotypic variance ( $\sigma_p^2$ ) =  $\sigma_g^2 + \sigma_e^2$

Genotypic Co-efficient of variation (GCV)

$$\text{GCV} = \frac{\sigma_g}{\mu} \times 100$$

Phenotypic Co-efficient of variation (PCV)

$$\text{PCV} = \frac{\sigma_p}{\mu} \times 100$$

#### Heritability

$$h^2_{(BS)} = \frac{\sigma^2_g}{\sigma^2_p} \times 100 \quad \text{or,}$$

$$h^2_{(BS)} = [(V_{F2} - V_{F1})/V_{F2}] \times 100$$

$$h^2_{(NS)} = \frac{\sigma^2_D}{\sigma^2_p} \times 100$$

$$V_P = V_g + V_e; V_e = (V_{P1} + V_{P2} + V_{F1})/3; V_g = V_{F2} - (V_{P1} + V_{P2} + V_{F1})/3$$

$h^2_{(BS)}$  - Heritability broad sense

$h^2_{(NS)}$  - Heritability narrow sense

$\sigma_D^2$  - Additive genetic variance

#### Genetic Advance (GA)

Improvement on mean genotypic value of selected plants over the base population is known as genetic advance under selection. It is the measure of genetic gain under selection.

$$\text{GA} = k\sigma_p h^2_{(BS)}$$

where,  $k$  = Selection differential which is constant for the known selection intensity

The value of  $k$  varies with the intensity of selection

Selection intensity (%)	Value of k
1	2.64
2	2.42

5	2.06
10	1.76
20	1.4
30	1.16

Selection intensity (%) =  $(q/n) \times 100$ ; where q - no. of plants selected, n – total no. of plants (base population)

### Interpretation of Result

1. **High Heritability and high genetic advance:** It indicate that additive genetic effect is there, and selection may be effective.
2. **High heritability and low genetic advance:** It indicate non additive gene action. The high heritability is being exhibited due to favourable influence of environment rather than genotype and selection for such trait may not be rewarding.
3. **Low heritability and high genetic advance:** It indicate that character is governed by additive gene effects. The low heritability is being exhibited due to high environmental effects. Selection may be effective in such cases.
4. **Low heritability and low genetic advance:** It indicate that the character is highly influenced by environmental effects and selection would be effective.

## RANDOMIZED BLOCK DESIGN

**Randomized Block Design (RBD):** A randomized block design is an **experimental design** where the experimental units are in groups called blocks. The treatments are randomly allocated to the experimental units inside each block. It is one of the most used experimental designs in plant breeding and extremely suitable for an experiment with 20-25 entries where the fertility gradient of the experimental area moves in one direction. The main features of this design are:

**Layout:** Experimental field is divided into homogeneous groups equal to the number of replications known as blocks. Then each block is further divided in to plots of similar shape and size equal to the number of treatments.

**Replication:** No restrictions on the number of replications. However, all the treatments should have equal number of replications.

**Randomization:** Treatments is allotted to the plots in each block by a random process. Separate randomization is used in each block.

**Local control:** The principle of local control is adopted in this design which control the experimental error by forming homogenous block.

### ANOVA table for Randomized Block Design (RBD).

Sources of variation	d.f.	S.S.	M.S.S.	S.S. / M.S.S.
Blocks	(r-1)			
Varieties	(t-1)			
Error	(r-1)(t-1)			
Total	(rt-1)			

Correction factor (CF) =  $(\text{Grand Total})^2 / \text{No. of observations}$

Total sum of squares =  $\sum x^2 - \text{CF}$

Treatment sum of squares =  $(\sum T^2/r) - \text{CF}$

Replication sum of squares =  $(\sum R^2/t) - \text{CF}$

Error Sum of squares = Total SS – Treatment SS – Replication SS

MSS = SS/d.f.

Genotypic variance =  $\frac{\text{MS due to treatment (genotype)} - \text{MS due to error}}{r}$

## HETEROSIS AND INBREEDING DEPRESSION

- Various types of heterosis are estimated as follows:

- Mid-parent Heterosis or Average Heterosis =  $\frac{F_1 - MP}{MP} \times 100$
- Heterobeltiosis Better Parent Heterosis =  $\frac{F_1 - BP}{BP} \times 100$
- Standard or economic heterosis =  $\frac{F_1 - CC}{CC} \times 100$
- Inbreeding Depression =  $\frac{F_2 - F_1}{F_1} \times 100$

Where,

F<sub>1</sub> = Mean value of a particular cross,

F<sub>2</sub> = Mean value of F<sub>2</sub> generation,

MP = Mean of two parents involved in the cross,

BP = Mean value of better parent of a cross,

CC = Mean value of a commercial cultivar or standard check

## DOUBLE CROSS HYBRIDS

The performance of double cross hybrids can be predicted by comparative evaluation of the predictions based on the performance of single cross. The method was developed by Jenkins (1934).

According to this method, the predicted performance of any double cross is the average performance of the four non-parental single crosses involving the four parental inbred.

**For example:** If the 4 inbred are  $I_1, I_2, I_3$  and  $I_4$ , the possible single cross among these inbred would be 6 viz.,  $I_1 \times I_2, I_2 \times I_3, I_3 \times I_4, I_1 \times I_3, I_1 \times I_4$  and  $I_2 \times I_4$ . These single crosses can combine to produce 3 double crosses, viz.,  $(I_1 \times I_2) \times (I_3 \times I_4), (I_1 \times I_3) \times (I_2 \times I_4)$  and  $(I_1 \times I_4) \times (I_2 \times I_3)$ . The performance of any of these double crosses can be predicted from the performance of the four single crosses, not involved in producing that particular double cross. For example: The performance of double cross  $(I_1 \times I_2) \times (I_3 \times I_4)$  would be the average of the performance of the four single crosses  $(I_1 \times I_3), (I_1 \times I_4), (I_2 \times I_3)$  and  $(I_2 \times I_4)$ .

If A, B, C and D are four inbreds then,

Prediction of double cross  $(A \times B) \times (C \times D) = [(A \times C) + (A \times D) + (B \times C) + (B \times D)] \frac{1}{4}$ .

## INDUCTION OF MUTATION

**Physical Mutagens:** Physical mutagens include various types of radiation, viz., X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultra-violet rays.

**Chemical Mutagens:** The chemical mutagens can be divided into four groups, viz. 1) alkylating agents, 2) base analogues, 3) acridine dyes, and 4) others.

Group of mutagens	Name of chemical	Mode of action
Alkylating Agents	Ethyl methane Sulphonate	AT to GC Transitions
	Methyl methane Sulphonate	Transitions
	Ethyl Ethane Sulphonate	GC to AT Transitions
	Ethylene Imines	Transitions
Base Analogues	5 Bromo Uracil	AT to GC Transitions
	2 Amino Purine	AT to GC Transitions
Acridine Dyes	Acridine, Proflavin	Deletion, addition and frame shifts
Others	Nitrous Acid	AT to GC Transitions
	Hydroxylamine	GC to AT Transitions
	Sodium Azide	Transitions

**Materials required:** Seed, pollen grain, buds, distilled water, X-ray, gamma ray, EMS, petri plates, conical flask, beaker, pipette, glass rods, measuring cylinder, stop watch, etc.

### Procedure:

1. Treat the seed or dormant propagating material with 10kR, 20kR, 40kR, 60kR, 80kR and 100kR of X-ray or gamma ray. Alternatively, treat the seed or propagating material with EMS at varying concentrations for 1, 2 and 4 hours.
2. Seeds may also be presoaked in the distilled water for different hours depending upon the seeds, to initiate biochemical reactions.
3. Intermittent shaking should be given to ensure uniform exposure of the chemicals. The chemical should be drained after the treatment time is over.
4. The seeds should be washed thoroughly in running tap water, immediately for not less than 30 minutes. After washing, the seeds should be dried in between the filter paper folds.
5. Do dense planting in the field along with a control plot.
6. Record observations on germination percentage, chlorophyll mutants and dominant mutants
7. Periodical observation on germination upto 10-15 days is needed. From the germination percentage, we can assess the LD50 dose.

## INDUCTION OF POLYPLOIDY

An organism or individual having more than two basic or monoploid sets of chromosomes is called polyploid and such condition is known as polyploidy.

When all the genomes present in a polyploid species are identical, it is known as autopolyploid but when two or more distinct genomes are present, it is known as allopolyploids.

**Materials required:** Seeds, colchicine, distilled water, cotton wool, beaker etc.

**Principle:** Colchicine is an alkaloid derived from the plant *Colchicum autumnale* is so far the best substance for inducing polyploidy. Pure colchicine is  $C_{22}H_{25}O_6N$ . Colchicine is non-toxic and water soluble. Colchicine is also soluble in alcohol,



chloroform and cold water but relatively less soluble in hot water. It produces a high frequency of polyploid cells. It can be applied in a range of concentration which may vary from 0.05 to 0.5 percent.

Colchicine prevents the formation of spindle fibre during the cell division in cells followed by non-disjunction of the chromosome at the equatorial plate, but division without moving to the poles. Thus, the chromosome complements are doubled in the cell. Since colchicine affects only dividing cells, it should be applied to a shoot-tip meristem only when its cells are actively dividing.

At any given time, only a small proportion of the cells would be in division; therefore, repeated treatments should be given at brief intervals to double the chromosome number in a large number of cells of the shoot apex. The polyploid and diploid cells present in a shoot-tip compete with each other and diploid cells may often out compete the polyploid ones. The degree of competition varies from species to species and even among varieties within species. Colchicine can be applied to the germinating seeds, coleoptiles, seedlings, sprouts, buds or shoots. The dose of the colchicine and duration of treatment vary with the crop as well as with the plant part which is to be treated.

### PLANTING OF A CROSSING BLOCK AND RAISING OF F<sub>1</sub> AND F<sub>2</sub> GENERATION

Procedure of planting a crossing block: A plant breeder for the improvement of a crop has many projects in hand at a time, such as breeding for high yield, disease, insect pest resistance, earliness, grain quality etc. There may be a set of varieties/lines, carefully chosen under each project for crossing inter se. It is advisable to grow all these parents together in a crossing block either in the glasshouse/greenhouse or in the field.

All the varieties/lines that are to be crossed do not flower at the same time, therefore, three to four sequential sowings of all the varieties/lines are done after every 7-10 days interval, so as to synchronize the flowering of very early and very late varieties/lines. Since emasculation and pollination techniques involve physical handling of plants, enough alleys or paths are provided between paired rows. All the paired rows are labelled. All the pollinated flowers are tagged. Name of pollen parent and date of crossing is mentioned on the tag. It is possible that while crossing, all the anthers may not be removed from some spikelets and the flower is tagged as crossed flower.

**Raising of F<sub>1</sub> generation:** A large number of F<sub>1</sub> seeds of different crosses are produced by a breeder every year. It is necessary to plant F<sub>1</sub> seeds of each cross along with the parents. If parents are homozygous, then all F<sub>1</sub> individuals of the cross would be genetically similar. In other words, F<sub>1</sub> is heterozygous but homogeneous. F<sub>1</sub> plants should not resemble the female parent for all the characters but should exhibit some characters of the pollen parent as well. Only then it is a true F<sub>1</sub> hybrid, otherwise it is a self-plant. F<sub>1</sub> seed of all crosses are spaced to obtain enough F<sub>2</sub> seed. The parents are also grown along with the F<sub>1</sub>s. This procedure allows the identification of any selfed plants in F<sub>1</sub> s in comparison with the parents.

**Raising of F<sub>2</sub> generation:** Seed harvested from F<sub>1</sub> plants are the F<sub>2</sub> seed and are sown next year. F<sub>2</sub> is the first segregating generation in which maximum variability is present, depending upon the genetic differences between the parents. A sizable population of F<sub>2</sub> (500 - 2000 plants) should be grown, so that most of the possible genotypes may be observed and effective selection can be practiced. The method of planting F<sub>2</sub> depends upon the procedure of handling this generation and the following generations. In case of pedigree method, F<sub>2</sub> seed are grown in widely spaced rows with an appropriate distance from plant to plant, so that each and every F<sub>2</sub> plant can be examined and selected or rejected. In bulk method, F<sub>2</sub> is planted just like a commercial crop and no selection is practiced. Parents of the cross as well as standard check varieties, upon which improvement is sought are also planted along with F<sub>2</sub> to aid in selection of desirable plants of different crosses.

### DATA COLLECTION AND RECORD KEEPING

In the breeding material and trials comprising germplasm, strains, varieties and segregating populations of various crosses, data are recorded on the qualitative and quantitative characters.

Data recorded on these traits are used for making comparisons among different experimental strains in various trials and are also used in inheritance studies.

A record is maintained of a complete history or pedigree of the various strains from the time of their introduction into a breeding programme up to maturity. Accurate record keeping enhances the efficiency of a breeder and way for effective selection.

Various types of record books maintained by a breeder

- i. **Accession record:** A record is maintained of all the plant material received and tested at an experiment station. For a crop, the accession number starts with unity each year. This number is preceded by the year in which a strain is first planted. For example, if a variety/strain is received in the year 2016, number of the first entry may be 1601. Thus, the accession number helps in identifying a strain and indicates the year in which it was first tested in a trial. An accession record gives information about the accession number, name of a variety/strain, source of seed, seed source number, pedigree, brief botanical description and remarks. It will be mentioned as EC = Exotic collection IC = Indigenous

collection.

- ii. **Diary of crosses:** It gives a brief history of the crosses for reference. Each diary starts with purpose of attempting the cross. Then information about date of attempting the cross, number of female spikes/flower buds pollinated, number of hybrid seed obtained, number of hybrid seed sown, number of  $F_1$  plants harvested, approximate number of  $F_2$  seed obtained, number of selections made etc. is recorded.
- iii. **Project book:** Each project on a breeding programme is numbered and a record of its name, year of start, purpose, breeding procedure followed and probable duration of the project is maintained.
- iv. **Sowing plan:** A sowing plan of each trial is prepared at the time of sowing. The plan provides the information on field measurements, row numbers in a plot, placement of check plots, direction of sowing and name of entries in a replication. Date of sowing, number of replications, gross plot size and net plot size are recorded on the top of each plan
- v. **Field notebooks:** These are used to record data on different characters relevant to each crop. These may be in the form of field registers or printed notebooks.